

Islet Assay using the XF24 Islet Capture Microplate

Introduction

XF Analyzers are most commonly used with an adherent monolayer of cells attached to an XF tissue culture plate. Many researchers, however, desire to use more physiologic cellular sources such as primary pancreatic islets which are routinely utilized to study diabetes. Seahorse Bioscience, in collaboration with the Mitochondrial ARC (Advancing Research through Collaborations) at Boston University School of Medicine, has developed a protocol that employs a novel consumable, the XF24 Islet Capture Microplate, to assess whole islet bioenergetics in vitro. The advantages over traditional methods (e.g. Clarke Electrode Apparatus) include higher throughput (20 sample per assay) and smaller amounts of pancreatic islets (as low as 30 islets per well).

Materials and Methods

The assay workflow (Figure 1) describes the procedure used to prepare reagent, materials and injected compounds.

The whole islet protocol described below is a modification of the XF24 Analyzer Protocol described in the XF24 User Manual (Version 1). Please feel free to modify the protocol to realize your intended research goals.

Modified XF Assay Media (MA Media): Supplement XF DMEM assay media with 3 mM glucose and 1% FBS to run whole islets. (FBS) is needed to prevent the islets from becoming too adherent.)

NOTE: When planning a Pancreatic Islet assay, Seahorse recommends using an anti-adherent for accurate reproducible results. Please contact Seahorse Technical Support with any questions.

Figure 1 | Pancreatic Islet Assay Workflow

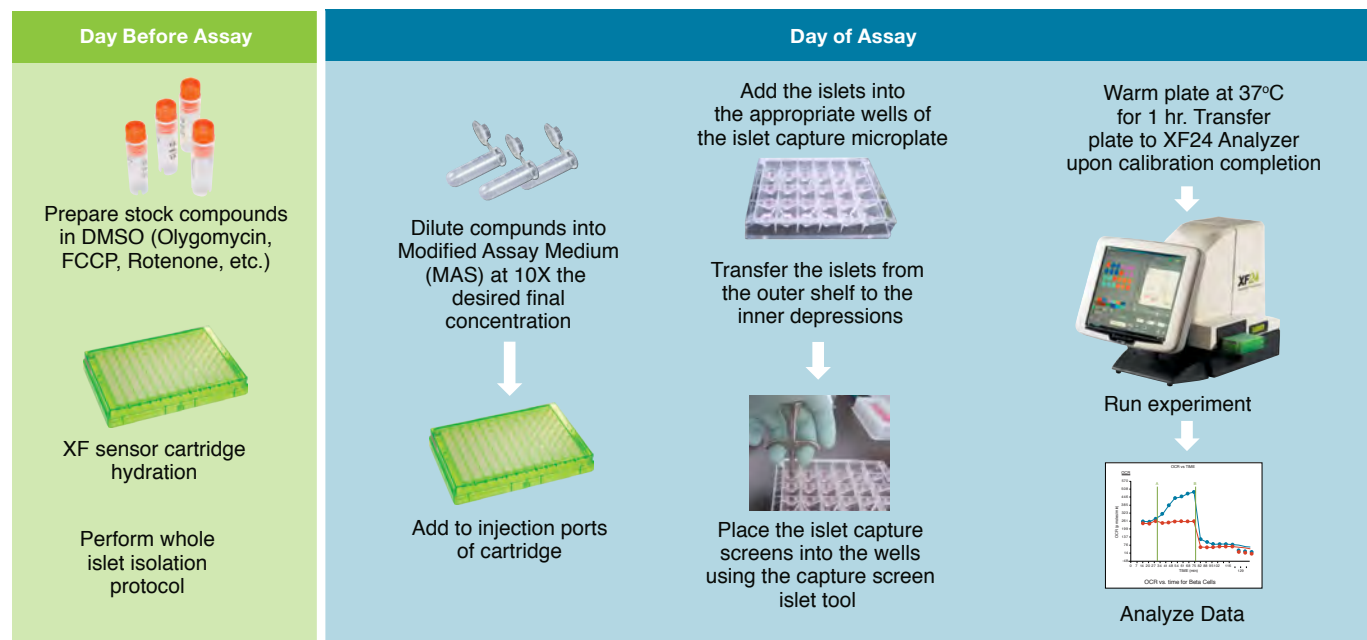


Table 2 | Components/Formulation of Modified XF Assay Media

Compound	Brand	Catalog Number	MW or Molar Concentration	Final Concentration	Grams or ml for 500 ml of XF Assay Media
Glucose	Sigma	G7528	180	3 mM	0.27 g
FBS	Hyclone	SH30070.03	100%	1%	5ml

It is recommended that all compounds to be added or injected are diluted into MA Media as described in Table 3.

Table 3 | Dilutions of Modified XF Assay Media

Compound	Brand	Catalog Number	Final Concentration	Dissolve in:
Rotenone	Sigma	R8875	5µM	Stock 1000X in DMSO. Dilute to 10X in MA Media.
Oligomycin	Sigma	O4876	5µM	Stock 1000X in DMSO. Dilute to 10X in MA Media.
FCCP	Sigma	C2920	1µM	Stock 1000X in DMSO. Dilute to 10X in MA Media.
Glucose	Sigma	G7528	20 µM	Stock 1000X in DMSO. Dilute to 10X in MA Media.
FBS	MP Biomedicals	155765	5µM	Methanol

Note: Oligomycin, FCCP, rotenone, and myxothiazol should be freshly diluted in MA Media for each experiment. Stock solutions in DMSO may be stored at -20°C.

Other items needed:

XF24 Sensor Cartridge

XF24 Islet Capture Microplate

Islet Capture Screens

Capture Screen Insert Tool

Calibration buffer (Seahorse Bioscience)

Dissecting microscope

Open faced bio-hood

Multi-channel pipettes and tips

Eppendorf and 15/50 ml Falcon tubes

Day Before the Assay:

1. Prepare an XF Assay Template (via the Assay Wizard)

- Using the XF24 Operation Manual as a guide and incorporating proper experimental design.
- Upload the assay template to the XF24 Analyzer before starting the assay. The experiment outlined here is an example of how to obtain the various mitochondrial respiration states using the XF24.
- Use Table 3 as a guide to program the Mix, Wait, Measure and Injection protocol.

2. Prepare the XF Sensor Cartridge

- Hydrate the XF Sensor Cartridge overnight in XF Calibration Buffer at 37°C, without CO₂
- Prepare whole islets by the standard protocol(s) used in your laboratory. For the protocol described here, ~8 mice were sacrificed to obtain ~1400 islets – enough for 20 wells at 70 islets/well. Incubate whole islets in a petri dish overnight under standard conditions for islet culture. (For the data shown, islets are cultured in RPMI media with 11 mM glucose, 10% FBS, and 1% pen/strep).

Table 3 | Typical Mix and Measurement Cycle times for XF24-3 assays

Command	Time (minutes)	Port
Calibration	–	–
Equilibrate	12*	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Inject A		
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Inject B		
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	

*Default equilibrate command consists of 2 min Mix, 2 min Wait repeated 3X. The same pattern could be followed for more injections.

Day of the Assay:

1. Add whole islets and capture screens to the wells.

- Aspirate islets from petri dish and dispense into a 50 ml tube.
- Wash 1X in MA Media.
- Remove supernatant and re-suspend in 2 ml MA Media.
- While creating turbulence in the tube with a 20 μ l pipettor, take 20 μ l aliquots and place as a drop on a culture dish – make 3 drops total (this gives you ~3% of the islets).
- Count islets under a dissecting microscope.

This will give you an average amount of islets per volume from which you can estimate the total number of islets.

- Determine the count of the islets, and adjust volume so you get ~70 islets for every 100 μ l of medial (700 islets/ml).
- Add 400 μ l MA Media to each well of the XF24 Islet plate.
- Add 50 μ l of the islet suspension to each well, and repeat so each well gets a total of 100 μ l of the islet suspension.

Final volume should be 500 μ l per well.

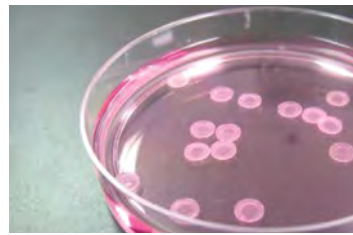
- When islets are seeded use a 20 μ l pipette to move all of the islets into the depressed chamber in the bottom of the well.

This step is tedious – use a dissecting microscope to be sure all of the islets are in the depression at the bottom of the well as in Figure 2.

2. Add screens by pre-wetting them in MA Media in a small Petri dish to remove any air bubbles. (See Figure 3)

- Use a pair of sterile forceps to position the screens so that the ring is facing up. (Figure 3)
- Use the capture screen insert tool to pick up an islet capture screen from the petri dish.
- Carefully place the islet capture screen in the bottom of each well using the capture screen insert tool. (Figure 4)

Figure 3 | Steps outlined in Day of Assay/Step 3



Face-up: Pre-wetting of screens to remove air bubbles.



Use capture screen Insert Tool

Press firmly into place to capture screen

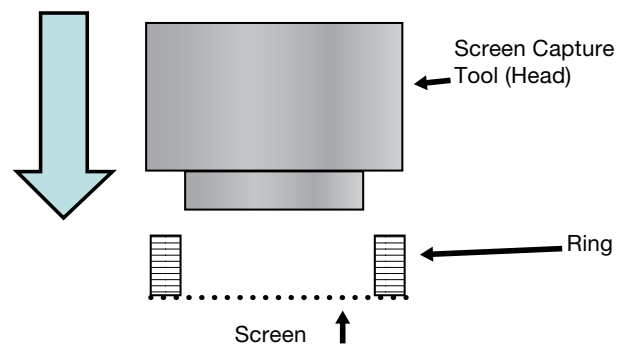
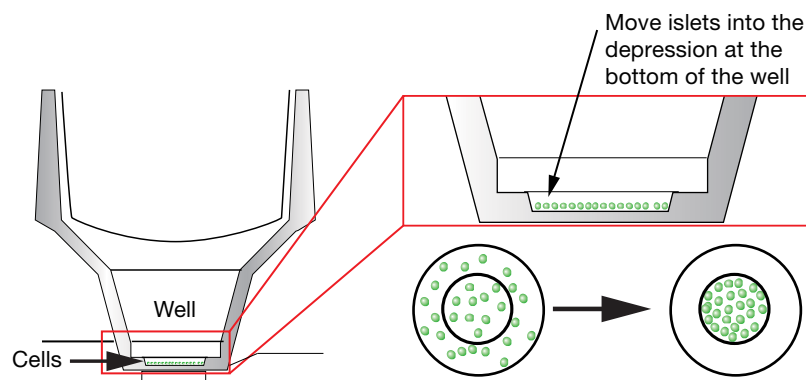


Figure 2 | Be sure all of the islets are in the depression at the bottom of the well.



- d. Take care during this step that you don't cause too much turbulence so as to keep the islets resting in the depression at the bottom of the well.
- e. Release the islet capture screen into the well by pulling up on the T-lever on the capture screen inset tool.
- f. Be sure the islet capture rings are stuck firmly at the bottom of the well. This can be confirmed by gently pushing the screen down with a blunt pipette tip. (Figure 5)
- g. Make sure that there is an islet capture screen in each well, even if there are no cells in the well. A microplate without a full complement of screens will cause problems with the head on the XF24 unit.

3. Run the Islet Capture Microplate on the XF24

- a. Place the microplate in an incubator set at 37°C, without CO₂.
- b. Store the microplate in the incubator for at least 1 h to equilibrate temp and adjust islet metabolism to 3 mM glucose.
- c. While plate is incubating, prepare cartridge with desired injections (See step 4).
- d. After cartridge is filled with compounds for injection, load the cartridge and start program and calibration.
- e. When the XF24 calibration is complete, place the islet plate into the XF24.

Run the program

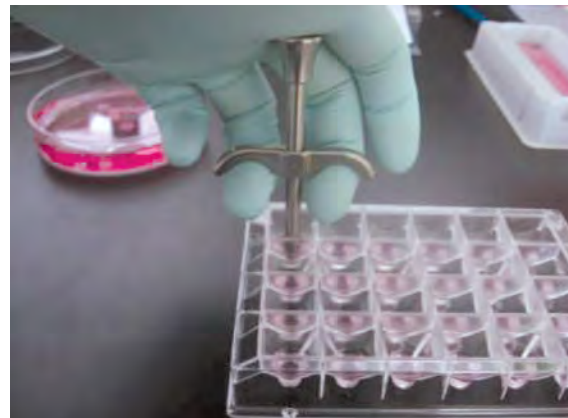
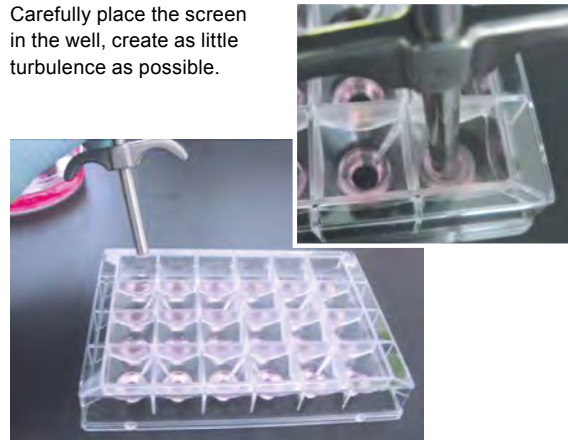
- f. After the program is complete you can normalize by counting the number of islets per well with the dissecting microscope. Islets may also be harvested for further downstream analysis, e.g. protein.
- g. Some users have found that this step was not necessary, as basal rates were sufficient for normalization.

4. Prepare Biosensor Cartridge with Injections and Calibrate

- a. Before calibration, load the XF sensor cartridge injection ports with following compounds listed in Table 4. (Next page bottom)
- b. Calibrate the sensor cartridge (loaded with desired compounds) as described in the XF manual.

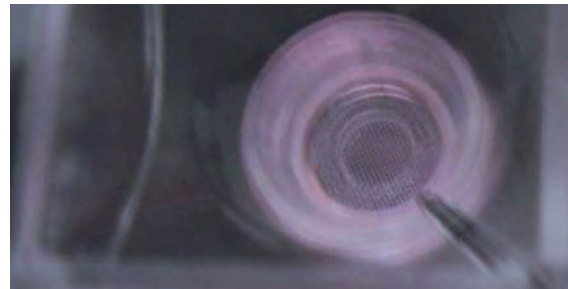
Figure 4 | Day of Assay/Place Islet Capture Screen

Carefully place the screen in the well, create as little turbulence as possible.

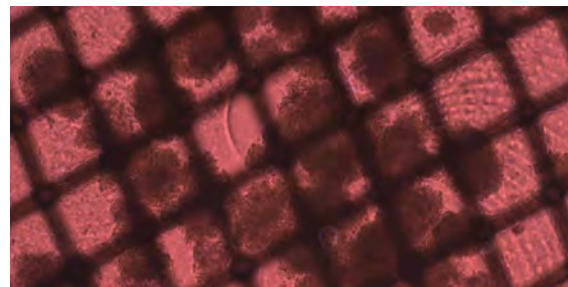


Pull up on the T-lever to release the capture screen once it's in place.

Figure 5 | Day of Assay/Checking the screen placement



Well with screen in place

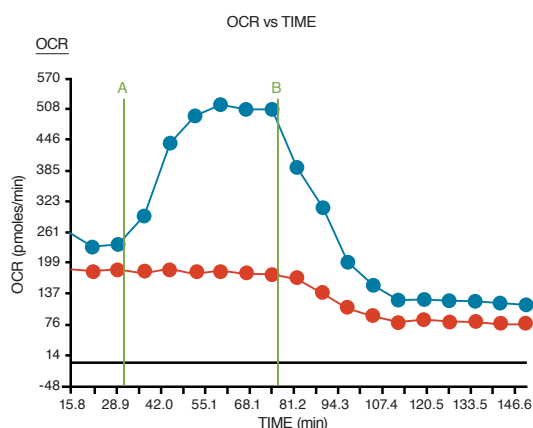


Islets under screen

Data Analysis

The results in tables 5 & 6 were obtained using 70 mouse islets/well or isolated beta cells.

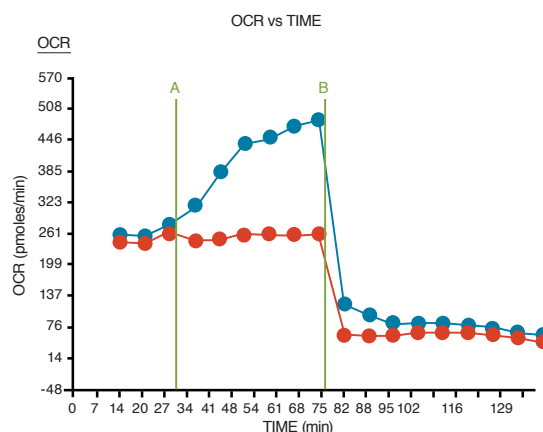
Table 5 | Oxygen Consumption Rate vs. time for Whole Islets



Whole pancreatic islets show a similar response to glucose addition as clonal INS1 beta cells run in a standard XF culture plate. Red lines – Blank injection at A; oligomycin at B. Blue lines – 20 mM glucose injection at A; oligomycin at B.

**Unpublished data from the Shirihai lab at Boston University School of Medicine*

Table 5 | Oxygen Consumption Rate vs. time for Beta Cells



Tables 7 & 8 show a direct comparison between normal human islets and diabetic human islets. Note that the basal OCR readings for the normal islets are 4X higher than that of diabetic islets and the response to glucose is depressed in the diabetic islets as compared to the normal islets.

Table 7 | Oxygen Consumption Rate vs. time for Normal Human Islets

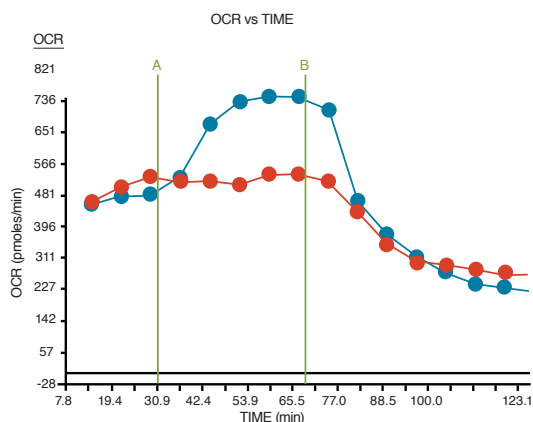
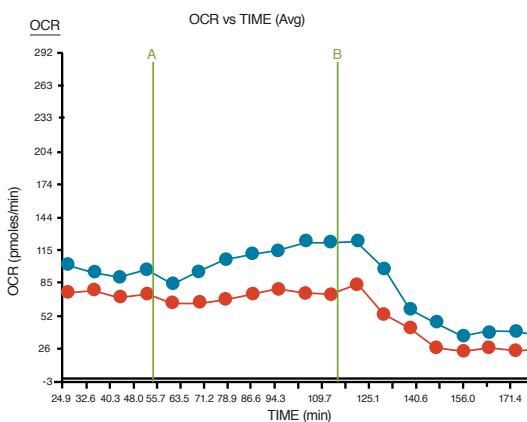


Table 8 | Oxygen Consumption Rate vs. time for Diabetic Human Islets



Comparisons of normal human islets versus diabetic human islets run in the Islet Capture Microplate. Red lines – Blank injection at A; oligomycin at B. Blue lines – 20mM glucose injection at A; oligomycin at B.

**Unpublished data from the Shirihai lab at Boston University School of Medicine*

Table 4 | XF sensor cartridge injection port compounds table

Injection Ports	Volume	Concentration in Port	Final Concentration in Well
Glucose	50 μ l	200 mM	20mM
Oligomycin	55 μ l	50 μ M	5 μ M
FCCP	60 μ l	10 μ M	1 μ M
Rotenone	65 μ l	50 μ M	5 μ M
Myxothiazol	65 μ l	50 μ M	5 μ M

Note: Vigorous mixing of the stock 20 μ M oligomycin is required to prevent precipitation. Rotenone and Myxothiazol are mixed together in the appropriate concentrations for injections.

Notes, Suggestions and Comments

The methods described above have been used successfully with whole pancreatic islets isolated from both mouse and humans. We believe that whole islets from other species can be used by following this protocol, however, the tissue, species (including age and sex), and method of isolation will contribute to the overall activity and other variables associated with the whole islets.

Starting values, ranges, and optimization: it is recommended that the following parameters be explored and optimized depending on the overall goal(s) of the experiment and research topic.

- Amount of whole islets per well
- The concentration of substrates and compounds injected
- Mix, Wait and Measure times.

References:

Please see Seahorse Bioscience's XF24 Training Course Workbook for a complete guide to operating and analyzing data used in the Seahorse XF24 Flux Analyzer Instrument.

For methods on isolating whole islets, please see: <http://www.jove.com/video/255/murine-pancreatic-islet-isolation>

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